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Study on the growth and tolerance ability of *Polygonum hydropiper* L. and *Hymenachne acutigluma* (Steud.) Gilliland on Pb and Cd polluted soil

Nghiên cứu khả năng chống chịu của nghể răm (Polygonum hydropiper L.) và bấc nhọn (Hymenachne acutigluma (Steud.) Gilliland) trên đất ô nhiễm chì và cadimi

Research article

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Two plant species including *Polygonum hydropiper* L. and *Hymenachne acutigluma* (Steud.) Gilliland were investigated in their resistance to lead (Pb) and cadmium (Cd) pollution in the soil. Lead-contaminated soil samples were collected from the lead recycling village Dong Mai, Chi Dao commune, Van Lam district, Hung Yen province, Vietnam that had Pb level up to 192,185 mg.kg^{-1}, dry weight (DW). Cadmium-contaminated soil samples were due to supplement of $CdCl_2.21/2H_2O$ to alluvial soil. Results showed that both species were highly resistant to Pb, however *P.hydropiper* was better. Similarly, the Cd resistance was higher for *P.hydropiper* than for *H.acutigluma*. No morpho-abnormalities of *P.hydropiper* regarding the impact of lead were recorded, whereas for *H.acutigluma*, the young leaves had white colour after two months of planting in soil containing lead levels of 192,185 mg.kg-1. The response of both species with Cd in soils included yellowing leaves, withering branches and even dying after 5-15 days exposed to Cd. Lead contents accumulated in above-ground parts of both plants were up to 4,650 and 3,161 mg.kg$^{-1,}$ DW, corresponding to *P.hydropiper* and *H.acutigluma*. From the research results on lead resistance and accumulation of two plant species studied, it is suggested that the two species are lead hyperaccumulators can be used for phytoremediation technology to clean contaminated soil.

Hai loài thực vật gồm nghể răm Polygonum hydropiper L. và bấc nhọn Hymenachne acutigluma (Steud.) Gilliland được nghiên cứu về khả năng chống chịu ô nhiễm chì (Pb) và cadmi (Cd) trong đất. Mẫu đất ô nhiễm chì được thu từ làng nghề tái chế chì Đông Mai, xã Chỉ Đạo, huyện Văn Lâm, tỉnh Hưng Yên, Việt Nam có hàm lượng chì lên đến 192.185 mg.kg^{-1}, tính theo trọng lượng khô (DW). Mẫu đất ô nhiễm Cd là do được bổ sung $CdCl_2.21/2H_2O$ vào đất phù sa. Kết quả cho thấy cả hai loài đều có sức chống chịu chì rất cao, tuy nhiên nghể răm có khả năng tốt hơn. Tương tự như vậy, sức chống chịu Cd của nghể răm cũng cao hơn của bấc nhọn. Không có dấu hiệu bất thường nào của nghể răm đối với tác động của chì được ghi nhận, trong khi ở bấc nhọn thì lá non có màu trắng sau hai tháng trồng trên đất có hàm lượng chì 192.185 mg.kg^{-1}. Phản ứng của cả hai loài thực vật với Cd trong đất gồm có hiện tượng vàng lá, héo ngọn và thậm chí chết sau 5-15 ngày phơi nhiễm với Cd. Hàm lượng chì được tích lũy cao trong phần trên mặt đất của cả hai loài thực vật lên tới 4.650 và 3.161 mg.kg^{-1}, DW, tương ứng cho loài P.hydropiper và H.acutigluma. Từ kết quả nghiên cứu về khả năng chống chịu và tích lũy chì của hai loài thực vật nghiên cứu, có thể đề xuất đây là hai loài siêu tích lũy chì có thể sử dụng trong công nghệ làm sạch đất ô nhiễm.

Keywords: heavy metal tolerance, lead and cadmium polluted soil, phytoremediation, *Polygonum hydropiper* L., *Hymenachne acutigluma* (Steud.) Gilliland

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1. Introduction

In Vietnam today, industrial production is being promoted and new industrial zones are being built up. Besides, the traditional handicraft villages causing the environment contaminated by toxic heavy metals in some areas have still existed.

The heavy metals mainly enter plant system through the soil or via the atmosphere (Arshad *et al.,* 2008; Uzu *et al.,* 2010). Among the heavy metals, lead is one of the most toxic and frequently encountered (Cecchi *et al.*, 2008; Grover *et al.*, 2010; Shahid *et al.*, 2011). This heavy metal has neuro-virulent properties that animals and human are very sensitive to. The toxic effect of lead is mainly causing a decrease or loss of activity of many enzymes that are important for cell functions (Peng et al., 2005).

The physics and chemistry methods have long been used to monitor and remediate environmental pollution giving good results. In addition, biology methods are considered friendly environmental technologies with low cost. Studies worldwide have shown that a number of plant species with high heavy metal accumulation capability can be used to treat heavy metal pollution. This method named phytoremediation has advantage like avoiding harmful effects on the lives and human health.

Since 1980s, scientists have begun to explore the use of plants to accumulate pollution for monitoring and cleaning up polluted water. Research on the tolerance of some plant species to heavy metals in order to find out potential species for indicator function as well as for phytoremediation contribute to improve the efficiency of heavy metal pollution assessment and treatment.

2. Materials and methods

2.1. Materials

There were two plants species investigated: *Polygonum hydropiper* L. belonging to the family Polygonaceae, and *Hymenachne acutigluma* (Steud.) Gilliland belonging to the family Poaceae.

Plant materials and soil samples (at the surface layer within 0 - 20 cm) that were polluted by Pb and Cd were collected at the lead recycling village Dong Mai belonging to Chi Dao commune, Van Lam district, Hung Yen province, Vietnam. Alluvial soils were collected in the Bio-Experimental Station in Co Nhue commune, Tu Liem district, Hanoi for being used as the control soil in some experiments.

2.2. Methods

The plant samples collected were divided into 2 parts for classification and lead (Pb) analysis for evaluation of their accumulation ability.

The scientific names of the plant species were determined by morphological classification method based on the documents as follows: Nguyen Thi Do (2007); Pham Hoang Ho (2000). The above ground parts (aerial tissues) of the plant samples were rinsed thoroughly with the tap water and re-rinsed with deionized water. Then they were dried at 60^oC in the oven (Memment-Germany) during 72 hours, ground and cut into very tiny pieces.

The soil samples were processed before being analysed by air-drying at room temperature for 5 - 7 days. The large-size debris, stones and pebbles were removed, after that the soil samples were ground and sieved through a 2 mm polyethylene sieve.

The mineralization of plant and soil samples was done with 65% HNO_3 solution (Merck-Germany) and some droplets of H_2O_2 at 100 - 110°C during 3 hours. The lead and cadmium contents were analysed by Atomic Absorption Spectroscopy (AAS). The accuracy of the analysis process was controlled by blank, duplication and reference samples (Tort-2 of Canada).

Conducting cultivation experiments in the tent with the plastic roof that allowed the sun shine goes through but rain water. The heavy metals Pb and Cd added to soil were prepared from soluble salts: $CdCl_2.21/2H_2O$ and $Pb(NO_3)_2$.

Assessment of heavy metal tolerance of plants was done based on their growth level (of the above ground parts only) on the polluted soil as well as abnormal signals.

Data were processed by using the excel program.

3. Results and discussion

3.1. Heavy metal tolerance and accumulation ability of plants collected in the lead recycling village

Two plants species were collected at two different points in Dong Mai village. *Polygonum hydropiper* L. was collected on the ditch bank at the distance of 50m in front of the lead smelters (96,456 mg $Pb.kg^{-1}$ soil, DW) *Hymenachne acutigluma* (Steud.) Gilliland was taken at the place having distance of 200m in front of the lead smelters (12,847 mg $Pb.kg^{-1}$ soil, DW).

These two plant species grew well on the soil heavily polluted by Pb without any abnormal signs. It is believed that only small amount of the lead in soil are soluble and available for plant uptake because of its binding with organic and/or colloidal materials (Kopittke *et al.,* 2008; Punamiya *et al.,* 2010). Therefore, it may be the reason that plants can grow in the soil seriously polluted by Pb. Beside the ability of tolerance with very high concentration of Pb in soil, these two plants accumulated Pb in their aerial parts up to 4,650 and 3,161 mg $Pb.kg^{-1}$, DW, respectively for *P.hydropiper* and *H.acutigluma*.

The accumulation of a high amount of Pb in the aerial parts of plants means the plants can extract well Pb from soil through their root systems then transfer to the upper parts. That accumulation characteristic of plants makes them can be used in the remediation process called phytoremediation.

For phytoremediation technology, an ideal plant species should have at least one of two following characteristics: very high metal accumulation capacity; high biomass yield with enhanced metal uptake potential (Friedland, 1990). This suggests that the suitable role of both plant species of our investigation in the cleaning up of lead contamination environment.

The concentration of Cd in that soil was low (0.2 mg $Cd.kg^{-1}$ soil, DW). However, plants could accumulate Cd into their above ground part at higher level. These values were 6.7 and 0.7 mg $Cd.kg^{-1}$, DW in *P.hydropiper* and *H.acutigluma*.

Many characteristic parameters were shown that have strong effects on the interactions of trace elements between soils and plants, therefore they influence on the efficiency of phytoremediation. For example, bioavailability of heavy metals in contaminated soils; soil texture; organic carbon content; cation exchange capacity; calcium carbonate equivalent; soil organisms; nutrient balance and pH-values (Saxena et al., 1999; Kamnev and Van der Lelie, 2000; Kabata-Pendias and Pendias, 2001). At acidic pH values, metals/heavy metals are more bioavailable. The organic matters and clays with high cation exchange capacities reduce metal availability and toxicity (Sinha and Sinha, 2008).

3.2. Heavy metal tolerance and growth ability of plants cultivated on the natural pollution soil in the tent

The specialized plastic bags were used in the culturing experiments; each of those contained 0.5 kg of soil. *P.hydropiper* and *H.acutigluma* were cultivated on the alluvial soil (refers to control, 0.16 mg $Cd.kg^{-1}$, DW) taken in the Bio-Experimental Station, and on natural pollution soil (192,185 mg $Pb.kg^{-1}$; 0.2 mg $Cd.kg^{-1}$, DW) taken in the lead recycling village, in order to assess their growth rate and tolerance potential to Pb and Cd in soil.

After one month of culturing, the two plant species were adapted to the new soil environment. This was considered as the adaptation period for them. Since then the height values of plant samples were measured every 5 days during 15 days. The data are shown in the table 1.

Table 1. Growth rate of *P.hydropiper* and *H.acutigluma* cultivated in alluvial soil and heavy metal polluted soil

Plant	Soil types	5 days		10 days		15 days	
		Compared to started point (cm)	Compared to control (%)	Compared to started point (cm)	Compared to control (%)	Compared to started point (cm)	Compared to control (%)
P.hydropiper	Alluvial soil	1.91	100	2.66	100	3.17	100
	Polluted soil	0.57	27	1.12	38.2	1.59	45.5
H.acutigluma	Alluvial soil	0.73	100	1.72	100	2.05	100
	Polluted soil	0.37	39.1	0.8	36.5	1.3	49.7

Figure 1. *H.acutigluma* after 2 months cultivated on the alluvial soil (left) and heavy metal polluted soil (right)

Two plant species showed no signs of being toxicity poisoned after 1 month of planting on heavy metal polluted soil taken in lead recycling village Dong Mai. The results in Table 1 clearly showed that when growing on alluvial soil, both of plant species had higher growth rates than plants growing on soil contaminated by Pb and Cd. After 15 days of observation and measurement, the height of control plants increased 2- 4 folds compared to that of plants in polluted soil. This proved that heavy metals especially Pb in the soil environment could be the one of factors that inhibited plant growth.

After two months of planting in the polluted soil, the young leaves of *H.acutigluma* had the white colour (Figure 1). This might be a sign of the plant stress to the toxicity of high levels of lead in addition to some other characteristics.

Of the two studied plant species, *P.hydropiper* is likely to endure greater pollution than *H.acutigluma*. It is shown that *P.hydropiper* expressed no signs of stress or toxicity as seen in *H.acutigluma*.

3.3. Heavy metal tolerance and growth ability of plants cultivated on the soil amended Pb and Cd in the tent

To assess more realistic Pb tolerance ability of two plant species, Pb polluted soil was added certain amounts of Pb to observe the growth rate and the response of the plants. In this kind of experiment, the polluted soil without amendment was considered as the control.

Table 2 shows the growth rate of two plant species planted in lead-contaminated soil with the addition of certain amounts of lead after 15 days.

Table 2. Growth rate of *P.hydropiper* and *H.acutigluma* cultivated in polluted soil with and without amendment of Pb after 15 days (n=12-15)

Soil (mg Pb.kg^{-1}, DW)	*P.hydropiper*		*H.acutigluma*	
	Compared to started point (cm)	Compared to control (%)	Compared to started point (cm)	Compared to control (%)
192,185 (control)	1.59±0.27	100	1.30±0.20	100
192,185 + 600	1.17±0.13	88.8	1.23±0.42	96.4
192,185 + 1,200	1.13±0.19	83.5	0.84±0.32	75.0
192,185 + 1,800	0.70±0.17	56.5	-	-

Both investigated plant species could grow on soil polluted heavily by Pb (192,185 mg.kg^{-1}), even when being added plus amounts of Pb (1,200 or 1,800 mg.kg^{-1}) in experimental period of 15 days. However, in the soil polluted with amendment of Pb, the growth rates of both two plant species were lower.

The effect of cadmium on the plant growth was also studied through the experiment of Cd amendment. Table 3 shows the growth rate of two plant species growing in alluvial soil with and without addition of certain amounts of cadmium after 15 days. The initial amount of Cd in the alluvial soil was very low (0.16 mg. kg^{-1}, DW) that is under the limitation given by Vietnam Standard (QCVN 03:2008).

Table 3. Growth rate of *P.hydropiper* and *H.acutigluma* cultivated in polluted soil with and without amendment of Cd after 15 days (n=8-10)

Soil (mg Cd.kg^{-1}, DW)	*P.hydropiper*		*H.acutigluma*	
	Compared to started point (cm)	Compared to control (%)	Compared to started point (cm)	Compared to control (%)
0.16 (control)	3.18±0.24	100	2.05±0.37	100
0.16 + 1,500	2.38±0.41	74.89	1.15±0.35	56.10
0.16 + 3,000	2.20±0.20	69.29	1.11±0.32	54.35
0.16 + 4,500	1.94±0.15	61.24	0.95±0.22	46.34

The height of plants cultivated in the soil added Cd increased but less than that of plant in control (Table 3). After 15 days of the experiment the growth rates of plants in Cd amended soil were only 61.24 to 74.89% in comparison to that in alluvial soil. High levels of cadmium had strong influence on the growth of *P.hydropiper*, even caused plant death (Figure 2).

Figure 2. *P.hydropiper* after 15 days cultivated on alluvial soil (right) and Cd amended soil (left)

After 7 days of the experiment, *P.hydropiper* in the formula added 3,000 mg.kg^{-1} occurred yellow leaves, and in formula supplemented 4,500 mg.kg^{-1} occurred yellow leaves together with withered branches. After 9 days of the experiment, leaf yellowing occurred also in the *P.hydropiper* in the formula added 1,500 mg.kg^{-1}. Plants started wilting and dying on the day 12 in the formula containing the highest content of Cd. After 15 days, *P.hydropiper* in formula supplemented Cd 3,000 mg.kg^{-1} had the highest proportion of dead individuals.

Figure 3. *H.acutigluma* after 15 days cultivated on alluvial soil (right) and Cd amended soil (left)

The increase rate of height of *H.acutigluma* cultivated in soil added Cd was significant lower than that in alluvial soil (Table 3), only equal to 46.34 - 56.1%. So the Cd tolerance ability of *H.acutigluma* was lower than that of *P.hydropiper*. This was also reflected in the signs of toxicity stress such as yellow leaf occurred shortly after 5 days of experiments in some plants of all the formulas added Cd. After 15-day-experiment, some *H.acutigluma* in the Cd supplemented formulas died (Figure 3).

4. Conclusion

Two investigated plant species have good resistance to high level of Pb in contaminated soil in the lead recycling village Dong Mai. They also can extract and store a large amount of Pb in their above ground parts. These two plant species can be the good candiate to be used in phytoremediation of Pb polluted soils. The Pb tolerance ability of *H.acutigluma* was lower than that of *P.hydropiper*. This is illustrated by the observed results that the young leaves of *H.acutigluma* had the white colour after two months of being planted in the soil containing very high concentration of Pb (192,185 mg $Pb.kg^{-1}$, DW).

For the soil cotaining Cd, especially soil added large amount of available form of Cd, two investigated plant species expressed their stress signals such as leaf yellowing, branch withering and even dying. The Cd tolerance ability of *H.acutigluma* was also lower than that of *P.hydropiper*. This was affirmed because lower growth rate of *H.acutigluma* in the Cd polluted soil as well as its yellow leaves and withered branches occurred earlier than *P.hydropiper*.

5. References

[1] Arshad M, Silvestre J, Pinelli E, Kallerhoff J, Kaemmerer M, Tarigo A, Shahid M, Guiresse M, Pradere P, Dumat C 2008. A fiel study of lead phytoextraction by various scented Pelargonium cultivars. Chemosphere 71 (11): 2187–2192.

[2] Cecchi M, Dumat C, Alric A, Felix-Faure B, Pradere P, Guiresse M 2008. Multi-metal contamination of a calcic cambisol by fallout from a lead-recycling plant. Geoderma 144 (1–2): 287–298.

[3] Friedland, A. G. 1990. The movement of metals through soils and ecosystems. In: Shaw, A. J. (ed) *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. CRC Press, Boca Raton, 7-19.

[4] Grover P, Rekhadevi P, Danadevi K, Vuyyuri S, Mahboob M, Rahman M 2010. Genotoxicity evaluation in workers occupationally exposed to lead. Int J Hyg Environ Health 213 (2): 99–106.

[5] Kabata-Pendias, A and Pendias, H. 2001. *Trace Elements in Soils and Plants*, 3rd Edition. CRC Press, Boca Raton, pp. 331.

[6] Kamnev, A. A. and Van der Lelie, D. 2000. Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. *Biosci Rep*., 20, 239-258.

[7] Kopittke PM, Asher CJ, Kopittke RA, Menzies NW 2008. Prediction of Pb speciation in concentrated and dilute nutrient solutions. Environ Pollut 153 (3): 548–554.

[8] Nguyen Thi Do, 2007. Flora of Vietnam. Vol. 11: Polygonaceae Juss. Science and Technology Publishing House: 185-186.

[9] Peng, H. Y., Tian, S. K., Yang, X. E. 2005. Changes of root morphology and Pb uptake by two species of Elsholtzia under Pb toxicity. Journal of Zhejiang University Science 6B(6):546-552.

[10] Pham Hoang Ho, 2000. An Illustrated Flora of Vietnam, Vol. III. TRE Publishing House, Hochiminh City, Vietnam: 668.

[11] Punamiya P, Datta R, Sarkar D, Barber S, Patel M, Das P 2010. Symbiotic role of glomus mosseae in phytoextraction of lead invetive rgrass [*Chrysopogon zizanioides* (L.)]. J Hazard Mater 177 (1–3): 465–474.

[12] QCVN 03: 2008/BTNMT. National technical regulation on the allowable limits of heavy metals in the soils.

[13] Saxena, P. K., KrishnaRaj, S., Dan, T., Perras, M. R. and Vettakkorumakankav, N. N. 1999. Phytoremediation of heavy metal contaminated and polluted soils. In: Prasad, M. N. V. and Hagemeyer, J. (eds.) *Heavy metal stress in plants: From molecules to ecosystems*. Springer Berlin Heidelberg, 305-329.

[14] Shahid M, Pinelli E, Pourrut B, Silvestre J, Dumat C 2011. Lead-induced genotoxicity to *Vicia faba* L. roots in relation with metal cell uptake and initial speciation. Ecotoxicol Environ Saf 74 (1): 78–84.

[15] Sinha, R. K. and Sinha, R. 2008 *Environmental Biotechnology*, Aavishkar, 315 pp.

[16] Uzu G, Sobanska S, Sarret G, Munoz M, Dumat C 2010. Foliar lead uptake by lettuce exposed to atmospheric fallouts. Environ Sci Techno l44: 1036–1042.